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Preliminary survey of the pinewood nematode in Turkey

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A survey was conducted in the northern conifer forests of Turkey in 2003 and 2004 for the pinewood nematode, *Bursaphelenchus xylophilus*. Wood samples were collected from declining pine trees, located in the following target areas: Düzce, Ankara, Bolu, and Artvin. Nematodes were extracted from over 310 samples and were observed and identified. *B. xylophilus* was not detected in any samples. *Bursaphelenchus* species were only found in 6% of the samples. From the *B. xylophilus*-group, only the species *B. mucronatus* was reported. Species identification was performed from morphological characters, particularly male spicules, and by molecular analysis with ITS-RFLP.

Introduction

Turkey is located on an area of transition between Europe and Asia. 26% of the country's land area is forests (21 million ha), of which only half is productive. Despite the enormous size of Turkey's forests, and their great biodiversity, Turkey imports large amounts of wood, importing almost 1 million m^3 of industrial coniferous wood in 1999 (DPT, 2001). The liberalization of international trade has increased the risk of introduction of nonindigenous species along with the increase in trade. Such species could harm countries' natural resources, and the goods and services which rely on them, thus countries face a new challenge of protecting their resources from this threat (Mack *et al*., 2000; Sakai *et al*., 2001).

The detection in Portugal of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970, marks one of the most recent introductions of an invasive species into pine forest ecosystems in Europe. This nematode is the causal agent of pine wilt disease (Mota *et al*., 1999) and is on the EPPO A1 list of pests recommended for regulation as quarantine pests. Following the detection of *B. xylophilus* in one of the member states of the European Union, specific measures to control and eradicate both the nematode and its insect vectors, *Monochamus* spp., from the affected area in Portugal were put into place in accordance with Directive 2001/219/EC. This directive also required surveys of *B. xylophilus* to be performed in other pine forests both within Portugal and in other members of the EU. Turkey, although not a member of the EU, has aligned its phytosanitary regulations with the EU ones.

The presence and distribution of *Bursaphelenchus* species in Turkey is poorly known. The first report of the genus *Bursaphelenchus* was made rather recently (Vieira *et al*., 2003) and knowledge about species present in Turkey is limited to several short reports (Vieira *et al*., 2004). In order to study the species of *Bursaphelenchus* associated with pine trees, in particular the members of the *xylophilus*-group, and to evaluate the potential risk of introduction into Turkey of *B. xylophilus*, a preliminary survey was conducted in 2003 and 2004 in several target areas in the northern shore region of Turkey.

Materials and Methods

In 2003–04 a survey was conducted in the pine forest areas of Ankara, Artvin, Düzce, Istanbul, Samsun and Trabzon, in Turkey. Wood samples, each 40–80 g in mass, were collected from pine trees (Table 1) displaying declining symptoms, 1.5 m up the trunk (DBH). A Pressler borer was used to sample from both sides of each tree, and samples were stored in polythene bags. Nematodes were extracted using a modified Baermann funnel technique, and processed within 48 h. The collected nematodes were inoculated on *Botrytis cinerea* growing in malt agar, and incubated for 2 weeks at 25°C.

For nematode identification, special attention was given to the group of species closely similar to *B. xylophilus* (*xylophilus*-group *sensu*; Ryss *et al*., 2005). Morphological identification was used to determine whether a species was part of the *B. xylophilus*-group, based on the following characters which define the species

Table 1 Number of wood samples, collected at each region, and respective host tree. The number of samples with *Bursaphelenchus* specimens is given in brackets

Host tree	Ankara	Artvin	Düzce	Istanbul	Samsun	Trabzon
Pinus brutia				9	6	
Pinus nigra	30 (2)		26 (6)	13	49	
Pinus pinaster			14 (1)	1		
Pinus pinea				3		
Pinus radiata			1			
Pinus sylvestris		65 (6)	18 (1)	15	15	46 (1)

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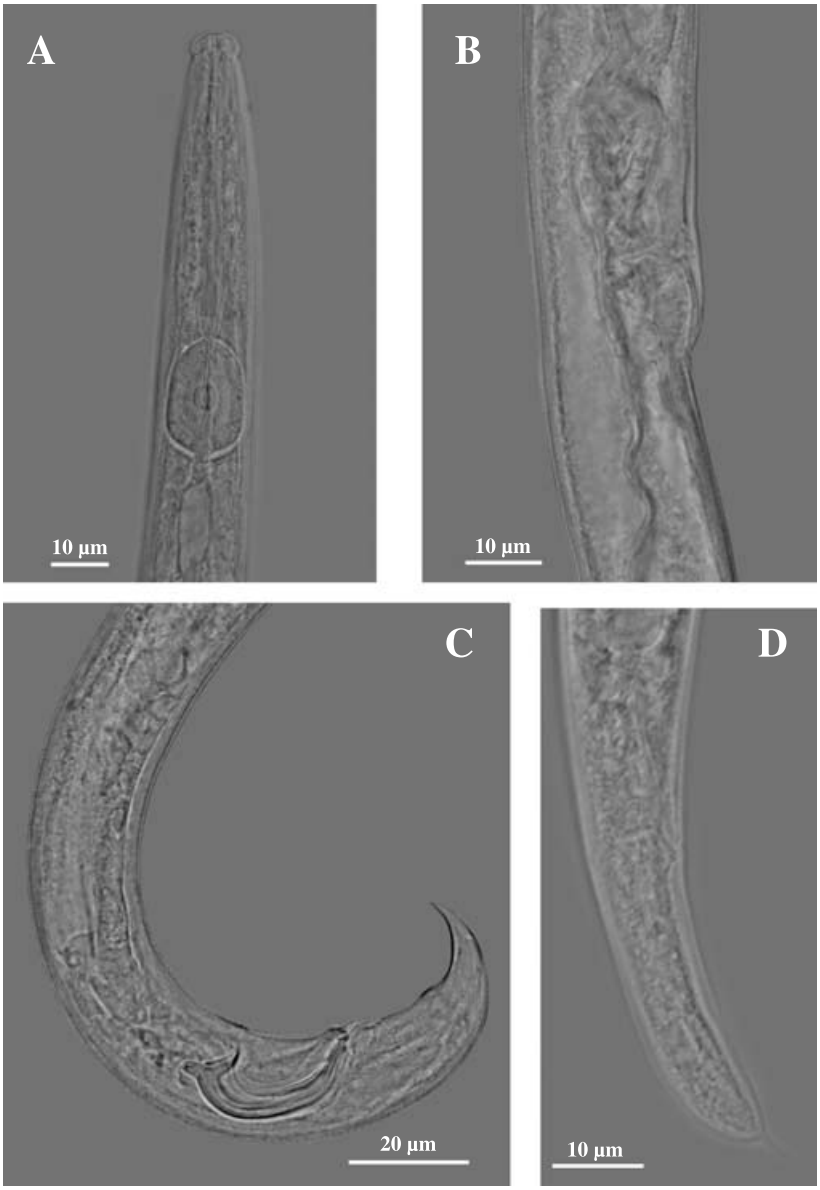

Fig. 1 Light optical microscope observations of *Bursaphelenchus mucronatus*. A: Anterior region; B: Vulva region; C: Male tail; D: Female tail.

group: the typical shape of the spicule: capitulum flattened anteriorly, condylus small, dorsal contour of lamina distinctly angular in last third, cucullus present (Fig. 1), and which are sufficient to separate this species from the other groups of *Bursaphelenchus* species (Ryss *et al*., 2005).

Where a nematode was identified as belonging to the *B. xylophilus*-group, identification was performed to species level, using morphological and molecular methodologies. For optical microscopic studies (Olympus BX50), nematodes were fixed with hot formalin (4%), processed to anhydrous glycerin and mounted in permanent slides according the 'express technique' described by Ryss (2003). The molecular analysis was performed following the methodology described in Cenis (1993) for DNA extraction, and the ITS-RFLP profiles were obtained following the methodology previously described in Hoyer *et al*. (1998) and Mota *et al*. (1999). Other *Bursaphelenchus* specimens were identified using only morphological methodologies, and only as far as the genus level.

Results

Only 17 of the 310 samples (6%) taken contained *Bursaphelenchus* specimens (Table 1). Further details about the geographical distribution of these 17 samples, the host trees from which the samples were taken, and the *Bursaphelenchus* species in the samples are summarized in Table 2.

Morphological identification identified several nematodes as belonging to one species in the *B. xylophilus*-group. These nematodes were analyzed molecularly with ITS-RFLP and compared with those described in Burgermeister *et al*. (2005),

Table 2 Host tree and localization of the *Bursaphelenchus* species found in north regions of Turkey

Sample No	Location	Tree	Stand type	*Bursaphelenchus* sp.
1	Ankara – ODTU campus	*P. nigra*	*P. nigra*	*Bursaphelenchus* sp. 1
2	Ankara – ODTU campus	*P. nigra*	*P. nigra*	*Bursaphelenchus* sp. 2
3	Artvin-Borçka	*P. sylvestris*	*P. sylvestris*	*B. mucronatus*
4	Artvin-Borçka	*P. sylvestris*	*P. sylvestris* + Spruce	*B. mucronatus*
5	Artvin-Borçka	*P. sylvestris*	Degraded *P. sylvestris*	*Bursaphelenchus* sp. 2
6	Artvin-Zeytinli	*P. sylvestris*	*P. sylvestris*	*Bursaphelenchus* sp. 2
7	Artvin-Zeytinli	*P. sylvestris*	*P. sylvestris* + Fir	*B. mucronatus*
8	Artvin-Zeytinli	*P. sylvestris*	*P. sylvestris* + Fir + Spruce	*B. mucronatus*
9	Düzce, Darıyeri	*P. nigra*	*P. nigra*	*B. mucronatus*
10	Düzce, Konutlar	*P. pinaster*	*P. pinaster*	*Bursaphelenchus* sp. 2
11	Düzce, Kurugöl	*P. nigra*	*P. nigra*	*B. mucronatus*
12	Düzce, Kurugöl	*P. nigra*	*P. nigra*	*B. mucronatus*
13	Duzce, Kurugöl	*P. nigra*	*P. nigra*	*B. mucronatus*
14	Düzce-Kurugöl	*P. nigra*	*P. nigra*	*B. mucronatus*
15	Düzce-Kurugöl	*P. nigra*	*P. nigra*	*B. mucronatus*
16	Düzce-Odayeri	*P. sylvestris*	Fir + *P. sylvestris* + Beech	*B. mucronatus*
17	Trabzon-Torul	*P. sylvestris*	*P. sylvestris*	*Bursaphelenchus* sp. 2

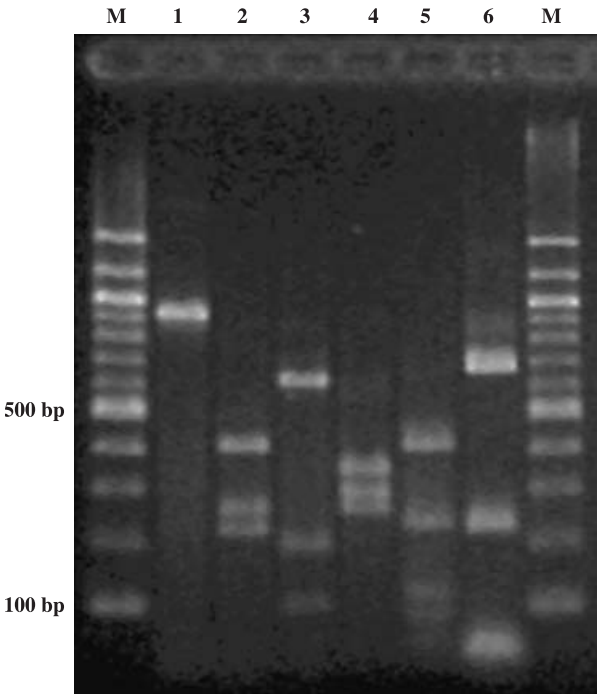

Fig. 2 ITS-RFLP patterns of *B. mucronatus*. Restriction fragments were obtained by digestion of the amplified rDNA fragment (1) with *Rsa I* (2), *Hae III* (3), *Msp I* (4), *Hinf I* (5) and *Alu I* (6). M: DNA size marker (100 bp ladder, Invitrogene).

and were clearly identified as *Bursaphelenchus mucronatus* Mamiya & Enda (1979) (Fig. 2). Other morphological characters, such as the female vulval flap and the presence of a mucron in the female tail, matched the description of the species *B. mucronatus* (Fig. 1).

Two other species, within the *B. pineperdae*-group *sensu* Ryss *et al*. (2005) were detected from wood samples (Table 2), and referred to as *Bursaphelenchus* sp. 1 and sp. 2, are still being identified.

Discussion

This preliminary study is the first survey for *B. xylophilus* in Turkey and did not detect the presence of this species in any pine wood sample from the surveyed areas. *B. mucronatus* was the only species of the *B. xylophilus*-group to be found, mainly in the regions of Düzce and Artvin. This species is widely distributed in Europe (mainly in the northern and central countries), some countries of Asia, and has been reported once from Canada (for a detailed distribution see Ryss *et al*., 2005).

The pathogenicity of *B. mucronatus* to different pine trees has been studied by several authors (Braasch, 1996; Mamiya, 1999; Caroppo *et al*., 2000; Kulinich, 2004; Michalopoulos-Skarmoutsos *et al*., 2004). Its pathogenic potential is still debatable according to some authors (Kulinich, 2004), while others state that this species, under natural conditions, is not a threat to forest ecosystems (Mamiya, 1999; McNamara, 2004). Besides that, *B. mucronatus* is one of the most abundant species in Europe and Asia, and no correlation or comparison has been made with the economic and natural impact caused by *B. xylophilus* in Portugal, or other Asian countries where this species has been reported as pathogenic to pine trees. Isolates of *B. mucronatus* found in Turkey, although associated with wilted or weakened pine trees, cannot be correlated with or established as the cause of the symptoms in, and ultimately the death of, pine trees.

Indigenous pine species (*P. nigra*, *P. sylvestris*) and the introduced species (*P. pinaster*) (DPT, 2001), the main species of conifer forests in Turkey, constitute a group of highly vulnerable trees which show high susceptibility to *B. xylophilus* (Evans

et al., 1996; McNamara, 2004). Other species (*P. brutia*, *P. radiata*) have been reported as associated host trees of the pinewood nematode (Ryss *et al*., 2005) in other countries.

The close similarity between the biology of *B. mucronatus* and that of *B. xylophilus*; the presence and distribution of *Monochamus galloprovincialis* (which has been identified as a vector of *B. xylophilus* in Portugal) in Turkey, mainly in northern, southern and western areas (Çanakçıoğlu & Mol, 1998; Özdikmen *et al*., 2005); and climatic conditions above 20°C in summer, define these areas as highly suitable for the possible establishment and survival *B. xylophilus* in Turkey.

A general survey for the pinewood nematode in these specific areas, the study of the *Bursaphelenchus* species present in the endemic and introduced pine species, as well as knowledge of other biological invading species are crucial to protect Turkish forests. Therefore, surveying for *B. xylophilus* in Turkey is continuing, in accordance with the recommendations of the EU and EPPO, and in order to study the distribution and biological importance of other *Bursaphelenchus* species across Turkey.

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Prospection préliminaire pour le nématode du pin *Bursaphelenchus xylophilus* en Turquie

Une prospection a été conduite dans les forêts de conifères du Nord de la Turquie en 2003 et 2004 pour détecter le nématode du pin, *Bursaphelenchus xylophilus*. Des échantillons de bois ont été collectés sur des pins mourants, situés dans les zones suivantes: Düzce, Ankara, Bolu, et Artvin. Des nématodes ont été extraits de plus de 310 échantillons et ont été observés et identifiés. *B. xylophilus* n'a été détecté dans aucun échantillon. Des espèces de *Bursaphelenchus* n'ont été trouvées que dans 6% des échantillons. Dans le groupe de *B. xylophilus*, seule l'espèce *B. mucronatus* a été signalée. L'identification des espèces a été réalisée à partir de caractères morphologiques, en particulier les spicules du mâle, et par analyse moléculaire par ITS-RFLP.

Предварительное обследование на выявление сосновой древесной нематоды в Турции

В 2003 и 2004 г. в хвойных лесах севера Турции проводилось обследование на выявление сосновой древесной нематоды *Bursaphelenchus xylophilus*. Образцы древесины отбирались с отмирающих сосен, находившихся в следующих областях: Дюцче, Анкара, Болу и Артвин. Нематоды экстрагировались из 310 образцов, подвергались наблюдению и идентификации. *B. xylophilus* не был обнаружен ни в одном из образцов. Виды рода *Bursaphelenchus* были найдены только в 6% образцов. Из группы *B. xylophilus* зарегистрирован только вид B. *mucronatus*. Идентификация видов выполнялась по морфологическим признакам, в частности по мужским спикулам, а также по молекулярным анализам с помощью ITS-RFLP.

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